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Coccidia (Apicomplexa: Eimeriidae) from Small Mammals of the Southwestern Sandhills in Nebraska, USA

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Abstract

Coccidian parasites of the genus *Eimeria* (Apicomplexa: Eimeriidae) were prepared and studied from small mammals collected from late July through early August during 2012 and 2013 on and around the area of the University of Nebraska-Lincoln, Cedar Point Biological Station, north of Ogallala, Nebraska. Habitats from which mammals were sampled included riparian, great-plains grasslands, and sandhills-grasslands. Twelve species representing 11 genera of small mammals were studied to begin to understand diversity of parasites in the area. Rodents of the following species were examined: *Geomys lutescens* Merriam, 1890; *Dipodomys ordii* Woodhouse, 1853; *Perognathus flavescens* Merriam, 1889; *Chaetodipus hispidus* (Baird, 1858); *Peromyscus leucopus* (Rafinesque, 1818); *Peromyscus maniculatus* (Wagner, 1845); *Reithrodontomys megalotis* (Baird, 1857); *Onychomys leucogaster* (Wied-Neuwid, 1841); *Microtus ochrogaster* (Wagner, 1842); *Zapus hudsonius* (Zimmerman, 1780); and *Spermophilus tridecimlineatus* (Mitchill, 1821). One species of shrew, *Blarina brevicauda* (Say, 1823), also was examined. *Eimeria* was found in 22 of 165 small mammals for an overall prevalence of 13%. A multivariate statistical analysis performed on measurement variables of the oocysts and sporocysts revealed reasonable separation of species of *Eimeria*.

Key words: biodiversity, canonical analysis, *Coccidia*, *Eimeria*, rodents, sandhills, small mammals

Introduction

From July through August of 2012 and 2013, as part of a field parasitology course conducted at the University of Nebraska-Lincoln, Cedar Point Biological Station (CPBS), individuals of several species of small mammals were collected from both the station grounds and from the surrounding area. The area surrounding CPBS is both geographically and ecologically diverse, providing opportunities for a wide range of biodiversity to be observed and studied. Little published information is available on the presence of parasites from mammals in the area, and no coccidia had been reported from mammals of this region. In the current study, 12 species of small mammals representing 11 genera were examined for presence of coccidia.

MATERIALS AND METHODS

Mammals examined for parasites were captured either with Sherman™ live-traps or Museum-Special™ snap-traps at or near Cedar Point Biological Station (see below for specific localities). All mammals collected and studied were either treated in chloroform (if they were from snap trap specimens) or killed in chloroform by vapor inhalation and examined for parasites within a few minutes after collection/death. Chloroform was used in both cases to kill ectoparasites that potentially could transfer to researchers/students at time of death of the small mammal. At necropsy, fecal pellets were removed from the lower bowel of each host and preserved in vials containing 2.0% aqueous (w/v) $K_2Cr_2O_7$. Up to the time of study, samples collected in 2012 were kept refrigerated at 3°C and those from 2013 were incubated at room temperature. Fecal samples were saved from 165 specimens. All samples were examined following the Sheather's (sugar) flotation method of Duszynski and Wilber (1997). Oocysts found were measured and imaged with a Zeiss Axiophot-II microscope equipped with both Neofluar and Nomarski-interference 63 x objective lenses; measurements were made with Zeiss™ software. All measurements were recorded in micrometers (µm). Measurements are provided in the Description of each species as the range, followed by mean, and standard deviation in parentheses. Specimens of mammals were deposited in the Division of Zoology, University of Nebraska State Museum, and both samples and images of parasites (cataloged as NP numbers) have been deposited in the Parasite Collection of the Harold W. Manter Laboratory of Parasitology, University of Nebraska State Museum.

Canonical discriminant analysis (CANDISC) of each species of *Eimeria* identified in this study was performed using the statistical package SAS 9.3. Measurements of five characters (oocyst length, oocyst width, sporocyst length, sporocyst width, and oocyst wall thickness) were made for each oocyst studied. Because of slight deviation from normality of measurements taken, all data were $\log_{10}$ transformed before analysis. Levels of statistical significance were set a-priori at $p \leq 0.05$. For all discriminant comparisons, Mahalanobis or generalized distances were used (Manly 1986).

RESULTS

Prevalance

Not all species of mammals examined were infected, but a summary of data for each species is provided in Table 1. Small mammals collected in 2012 were found to harbor oocysts of the genus *Eimeria* in 6 of 81 (7.2%) specimens. Oocysts of *Eimeria* were found in 16 of 84 (19%) specimens examined in 2013 from the same localities. Cumulative prevalence for both years was 13.3% (22 of 165 specimens).

Descriptions

Measurements of oocysts of five species of *Eimeria* collected from seven species of mammals are provided in Table 2. Prior to this study, no data were available on coccidian parasites of small mammals of the southwestern Sandhills of Nebraska; therefore, we present below complete accounts of *Eimeria* from each species of infected mammal that we studied. Photographs of oocysts from each host species also are provided.

Host: *Peromyscus leucopus* (Rafinesque, 1818)

Eimeria arizonensis Levine et al. 1957

Description.—Oocyst wall smooth and 1.5 (±0.2) thick. Sporulated oocysts 26.1–29.9 by 21.7–23.8, 28.2 (±1.3) by 22.7 (±0.8) and a length:width ratio of 1.2 (±0.1). Sporocysts 10.1–13.3 by 7.7–8.7, 11.9 (±1.3) by 8.4 (±0.3) and a length:width ratio of 1.5 (±0.2). See Figures 1 and 2.

Prevalence.—E. arizonensis was found in eight of 39 (20.5%) animals examined.

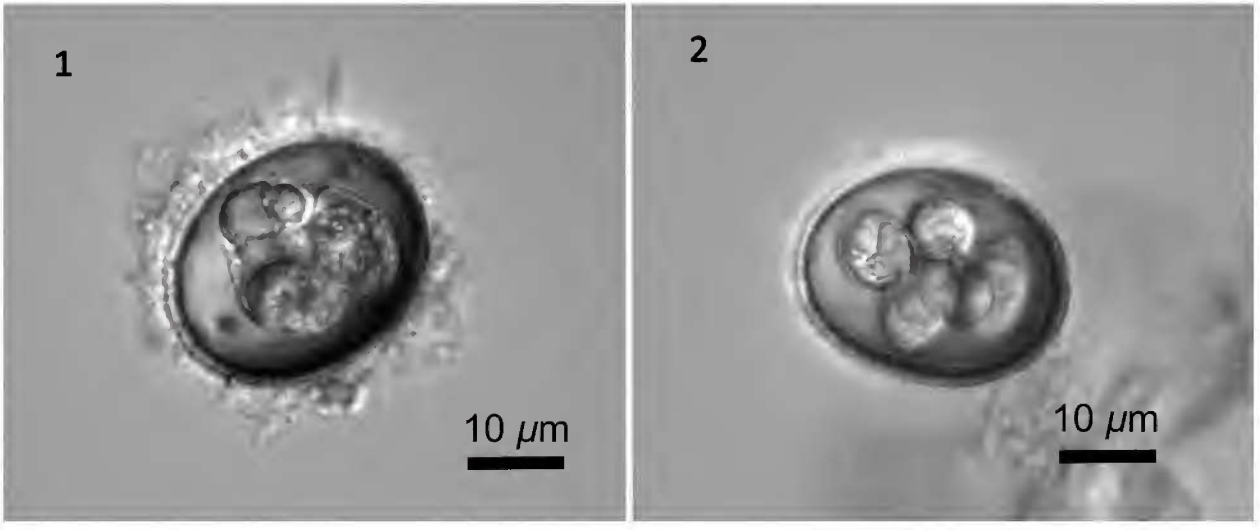
Specimens deposited.—NP1364, NP1374, NP1387, NP1407, NP1408, NP1411, NP1446, and NP1474.

Table 1. Prevalence of *Eimeria* in small mammals collected in western Nebraska during 2012 and 2013. Data presented as: positive individuals/total individuals (% positive).

Host	2012	2013	Total
Blarina brevicauda	0/1 (0)	-	0/1 (0)
Chaetodipus hispidis	1/11 (9)	-	1/11 (9)
Dipodomys ordii	1/3 (33)	0/4 (0)	1/7 (14)
Geomys lutescens	-	0/7 (0)	0/7 (0)
Microtus ochrogaster	0/8 (0)	0/2 (0)	0/10 (0)
Onychomys leucogaster	1/8 (13)	1/2 (50)	2/10 (20)
Perognathus flavescens	1/8 (13)	1/6 (17)	2/14 (14)
Peromyscus leucopus	0/1 (0)	8/38 (23)	8/39 (21)
Peromyscus maniculatus	1/31 (3)	3/8 (38)	4/39 (10)
Reithrodontomys megalotis	1/9 (11)	3/13 (23)	4/22 (18)
Spermophilus tridecemlineatus	0/1 (0)	-	0/1 (0)
Zapus hudsonius	-	0/4 (0)	0/4 (0)
Total	6/81 (7)	16/84 (19)	22/165 (13)

Location.—Gastrointestinal tract. Oocysts obtained from fecal sample.

Locality.—Infected White-footed Mice were trapped on eight occasions at the following localities: 23 July 2013 [CPBS] (41°12′29″N, 101°38′42″W); 23 July 2013 [CPBS] (41°12′40″N, 101°39′12″W); 24 July 2013 [CPBS] (41°11′36″N, 101°50′03″W); 24 July 2013 [CPBS] (41°12′37″N, 101°39′11″W); 26 July 2013 [CPBS] (41°12′37″N, 101°38′56″W); 28 July 2013 [CPBS] (41°12′37″N, 101°38′56″W); 31 July 2013 [Ackley Valley, Haythorn Ranch, Arthur County, NE] (41°12′00″N, 101°26′06″W); 5 August 2013 [Breen's Flyway] (41°10′53″N, 101°21′32″W).



Figures 1 and 2. Photomicrographs of sporulated oocysts of *Eimeria arizonensis* recovered from the feces of *Peromyscus leucopus*.

Table 2. Measurements of oocyst characters of five species of *Eimeria* from small mammals collected in western Nebraska (2012–2013). Data provided in the following form: mean, range, standard deviation, and coefficient of variation (%).

Eimeria species	Host species	Oocyst length	Oocyst width	Oocyst length:width ratio	Sporocyst length	Sporocyst width	Sporocyst length:width ratio	Oocyst wall width	Wall structure
E. arizonensis	*Reithrodontomys megalotis*	23.3, 18.9–28.8, 2.7, 11.5	20.3, 15–23.1, 1.8, 8.9	1.2, .9–1.6, 0.1, 10.6	11.1, 8–13.4, 1.5, 13.7	7.8, 5.9–9.3, 0.7, 8.8	1.4, 1.1–1.8, 0.1, 12.6	1.2, 1.0–1.6, 0.13, 11.1	Smooth
E. arizonensis	*Peromyscus leucopus*	28.2, 26.1–29.9, 1.3, 4.5	22.7, 21.7–23.8, 0.8, 3.4	1.2, 1.2–1.4, 0.1, 5.0	11.9, 10.1–13.3, 1.3, 10.5	8.4, 7.7–8.7, 0.3, 4.0	1.4, 1.3–1.6, 0.1, 8.3	1.5, 1.2–1.8, 0.2, 13.8	Smooth
E. arizonensis	*Peromyscus maniculatus*	27.7, 27.3–28.7, 0.4, 1.4	22.8, 21.8–24.1, 0.5, 2.2	1.2, 1.2–1.3, 0.02, 2.0	13.3, 12–14.9, 0.7, 5.0	7.5, 6.2–8.5, 0.5, 7.0	1.8, 1.5–2.1, 0.15, 8.6	1.5, 1.3–1.8, 0.15, 9.7	Rough
E. balphae	*Dipodomys ordii*	15.6, 14.1–17, 0.83, 5.3	13.9, 12.9–15.3, 0.7, 4.9	1.1, 1 -1.3, 0.1, 8.1	7.9, 7.4–8.6, 0.4, 5.1	5.1, 4.7–5.6, 0.3, 6.4	1.6, 1.4–1.7, 0.1, 4.6	1.2, 1.0–1.3, 0.15, 9.7	Smooth
E. hispidensis	*Chaetodipus hispidis*	20.8, 19.4–22.1, 0.9, 4.3	18.1, 17.2–19.7, 0.9, 5.0	1.1, 1.1–1.3, 0.1, 4.5	9.3, 7.8–9.8, 0.7, 7.5	7.1, 6.5–7.6, 0.4, 6.1	1.3, 1.2–1.5, 0.1, 8.0	1.1, 0.9–1.2, 0.1, 9.1	Smooth
E. onychomysis	*Onychomys leucogaster*	20.1, 19.2–22, 0.6, 3.0	16.5, 17.2–19.7, 0.9, 5.0	1.2, 1.1–1.3, 0.05, 3.8	9.8, 6.8–12.1, 1.4, 14.4	7.0, 5.1–7.8, 0.6, 8.6	1.4, 1.0–1.8, 0.19, 13.7	1.1, 0.7–1.3, 0.1, 12.4	Smooth
E. sp.	*Perognathus flavescens*	21.8, 18–24.6, 2, 9.4	18.6, 14.1–21.4, 1.8, 9.6	1.2, 1–1.3, 0.05, 4.5	8.8, 7.5–10.3, 0.7, 8.2	7.4, 6.3–8.7, 0.7, 8.9	1.2, 1.0–1.3, 0.06, 5.0	1.2, 0.8–1.4, 0.17, 14	Rough

Host: *Peromyscus maniculatus* (Wagner, 1845)

Eimeria arizonensis

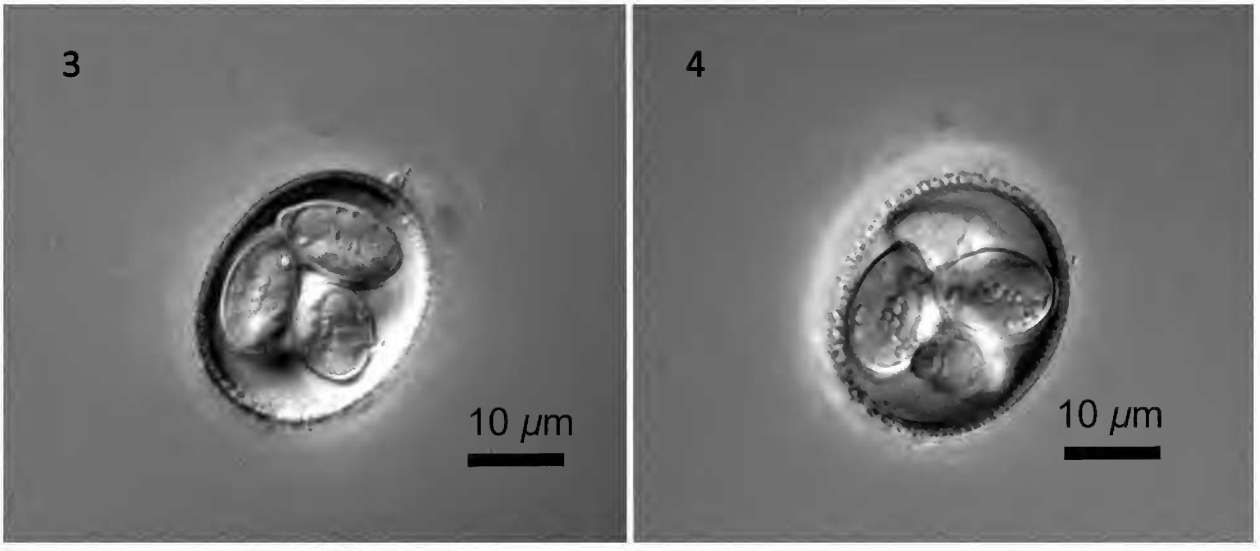
Description.—Oocyst wall rough and 1.5 (±0.15) thick. Sporulated oocysts 27.3–28.7 by 21.8–24.1, 27.7 (±0.4) by 22.8 (±0.5) and a length:width ratio of 1.2 (±0.02). Sporocysts 12.0–14.9 by 6.2–8.5, 13.3 (±0.7) by 7.5 (±0.5) and a length:width ratio of 1.8 (±0.15). See Figures 3 and 4.

Prevalence — This species was found in 4 of 39 (10.2%) animals examined.

Specimens deposited.—NP1251, NP1353, NP1407, and NP1418.

Location.—Intestine. Oocysts obtained from fecal sample.

Locality.—Infected species of this mouse were trapped on four separate occasions at the following localities: 26 July 2013 [Ackley Valley, Haythorn Ranch, Arthur County, NE] (41°12′00″N, 101°26′06″W); 23 July 2012 and 30 July 2013 [2.4 km south of CPBS in the area known locally as the grama grass locality] (41°11′38″N, 101°38′56″W); 23 July 2013 [CPBS] (41°12′34″N, 101°38′57″W).



Figures 3 and 4. Photomicrographs of sporulated oocysts of *Eimeria arizonensis* recovered from the feces of *Peromyscus maniculatus.*

Host: *Reithrodontomys megalotis* (Baird, 1857)

Eimeria arizonensis

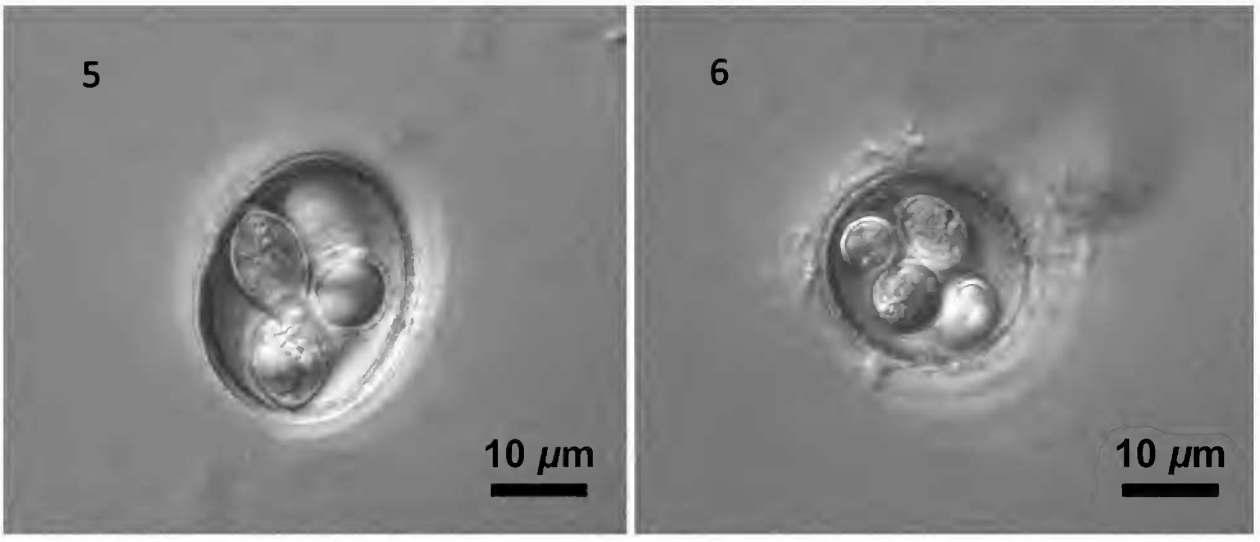
Description.—Oocyst wall smooth and 1.2 (±0.13) thick. Sporulated oocysts 18.9–28.8 by 15–23.1, 23.3 (±2.7) by 20.3 (±1.8) and a length:width ratio of 1.2 (±0.1). Sporocysts 8–13.4 by 5.9–9.3, 11.1 (±1.5) by 7.8 (±0.7) and a length:width ratio of 1.4 (±0.1). See Figures 5 and 6.

Prevalence.—This species was found in four of 22 (18.2%) animals examined.

Specimens deposited.—NP1224, NP1475, NP1490, and NP1510.

Location.—Intestine. Oocysts obtained from fecal sample.

Locality.—Infected Western Harvest Mice were trapped on four occasions at the following localities: 18 July 2012 [CPBS] (41°12′34″N, 101°38′57″W); 5 August 2013, 6 August 2013, 7 August 2013 [Breen's Flyway] (41°10′53″N, 101°21′32″W).



Figures 5 and 6. Photomicrographs of sporulated oocysts of *Eimeria arizonensis* recovered from the feces of *Reithrodontomys megalotis*.

Host.—*Dipodomys ordii* Woodhouse, 1853

Eimeria balphae Ernst et al., 1967

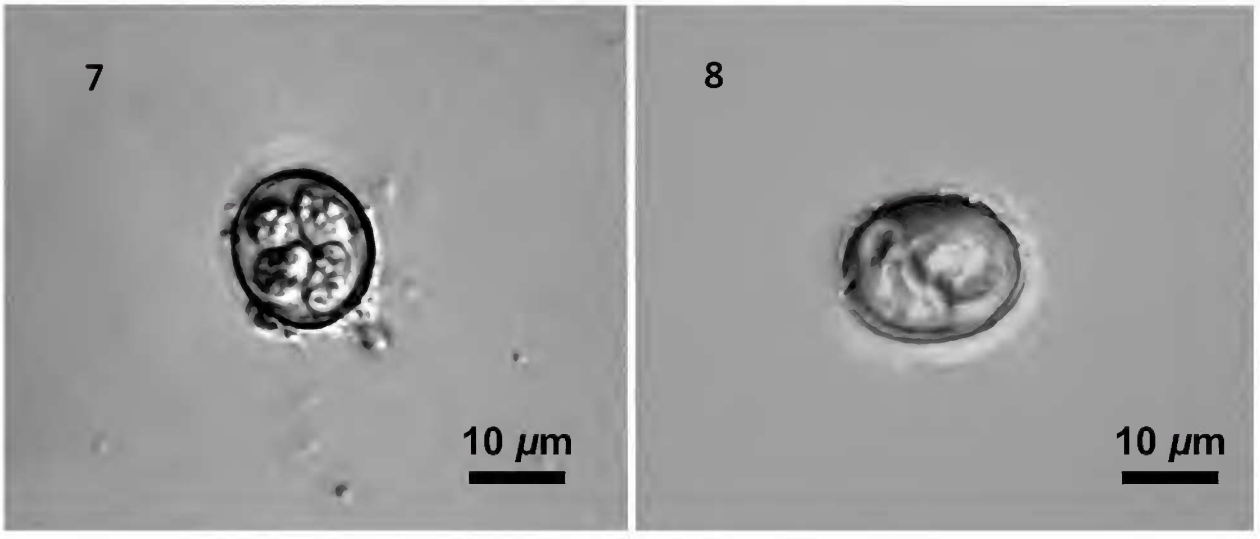
Description.—Oocyst wall smooth 1.2 (±0.15) thick. Sporulated oocysts 14.1–17 by 12.9–15.3, 15.6 (±0.83) by 13.9 (±0.7) and a length:width ratio of 1.1 (±0.1). Sporocysts 7.4–8.6 by 4.7–5.6, 7.9 (±0.4) by 5.1 (±0.3) and a length:width ratio of 1.6 (±0.1). See Figures 7 and 8.

Prevalence.—This species was found in one of seven (14.3%) animals examined.

Specimen deposited.—NP1229.

Location.—Intestine. Oocysts obtained from fecal sample.

Locality.—This infected kangaroo rat was collected on 19 July 2012 [east of CPBS] (41°12′37″N, 101°38′56″W).



Figures 7 and 8. Photomicrographs of sporulated oocysts of *Eimeria balphae* recovered from the feces of *Dipodomys ordii*.

Host.—*Chaetodipus hispidus* (Baird, 1858)

Eimeria hispidensis Ford et al. 1990

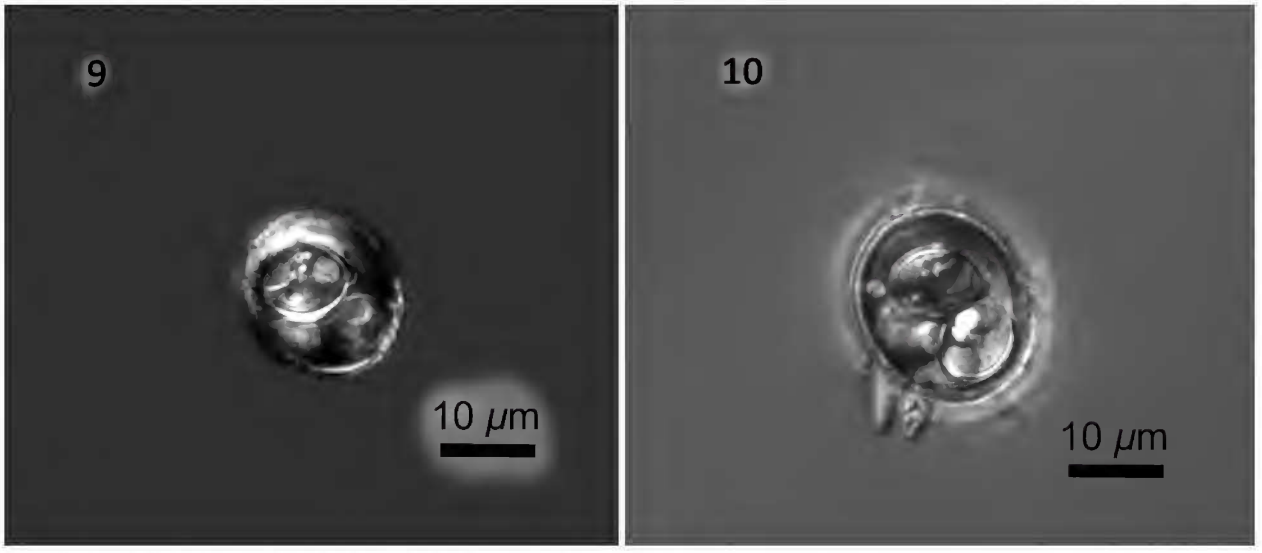
Description.—Oocyst wall smooth and 1.1 (±0.1) thick. Sporulated oocysts 19.4–22.1 by 17.2–19.7, 20.8 (±0.9) by 18.1 (±0.9) and a length:width ratio of 1.1 (±0.1). Sporocysts 7.8–9.8 by 6.5–7.6, 9.3 (±0.7) by 7.1 (±0.4) and a length:width ratio of 1.1 (±0.1). See Figures 9 and 10.

Prevalence.—This species was found in one of 11 (9%) animals examined.

Specimens deposited.—NP1272.

Location.—Intestine. Oocysts obtained from fecal sample.

Locality.—The infected Hispid Pocket Mouse was trapped on 25 July 2012 [2.4 km south of Cedar Point Biological Station in the area known locally as grama grass locality] (41°11′38″N, 101°38′56″W).



Figures 9 and 10. Photomicrographs of sporulated oocysts of *Eimeria hispidensis* recovered from the feces of *Chaetodipus hispidis*.

Host.—*Onychomys leucogaster* (Wied-Neuwid, 1841)

Eimeria onychomysis Levine et al. 1957

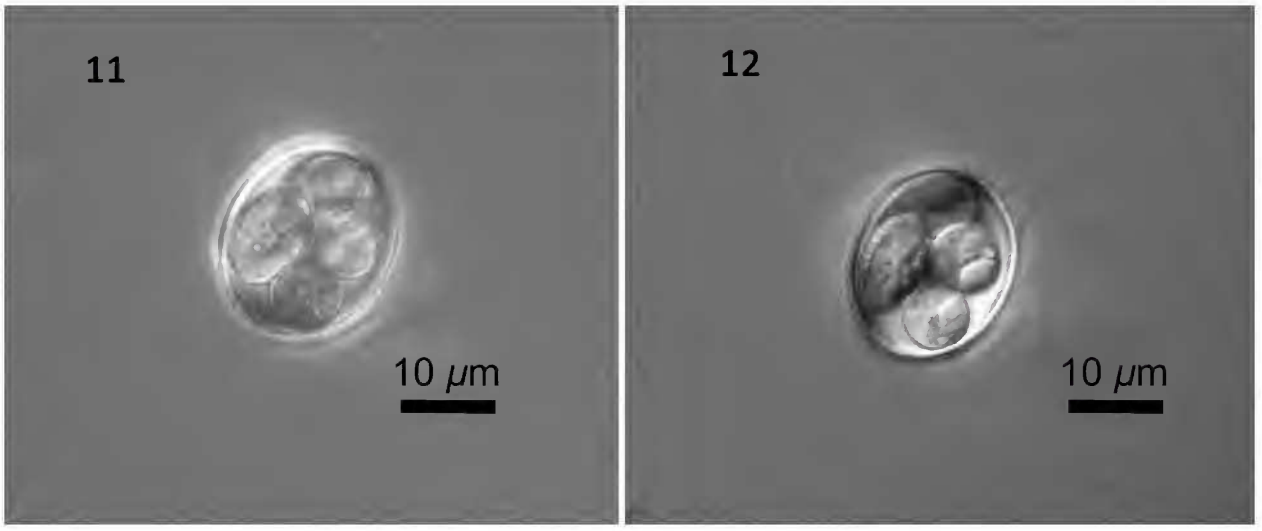
Description.—Oocyst wall smooth 1.1 (±0.1) thick. Sporulated oocysts 19.2–22.0 by 17.2–19.7, 20.1 (±0.6) by 16.5 (±0.9) and a length:width ratio of 1.2 (±0.05). Sporocysts 6.8–12.1 by 5.1–7.8, 9.8 (±1.4) by 7.0 (±0.6) and a length:width ratio of 1.1 (±0.05). See Figures 11 and 12.

Prevalence.—This species was found in two of 10 (20%) animals examined.

Specimens deposited.—NP1259 and NP1426.

Location.—Intestine. Oocysts obtained from fecal sample.

Locality.—Infected Northern Grasshopper Mice were trapped on two occasions: 30 July 2013 [Ackley Valley, Haythorn Ranch, Arthur County, NE] (41°12′00″N, 101°26′06″W) and 23 July 2012 [2.4 km south of CPBS in the area known locally as the grama grass locality] (41°11′38″N, 101°38′56″W).



Figures 11 and 12. Photomicrographs of sporulated oocysts of *Eimeria onychomysis* recovered from the feces of *Onychomys leucogaster*.

Host.—*Perognathus flavescens* Merriam, 1889

Eimeria sp. (unidentified species)

Description.—Oocyst wall rough and 1.2 (±0.17) thick. Sporulated oocysts 18–24.6 by 14.1–21.4, 21.8 (±2.0) by 18.6 (±1.8) and a length:width ratio of 1.2 (±0.05). Sporocysts 7.5–10.3 by 6.3–8.7, 8.8 (±0.7) by 7.4 (±0.7) and a length:width ratio of 1.2 (±0.06). See Figures 13-16.

Prevalence.—This species was found in two of 14 (14.3%) animals examined.

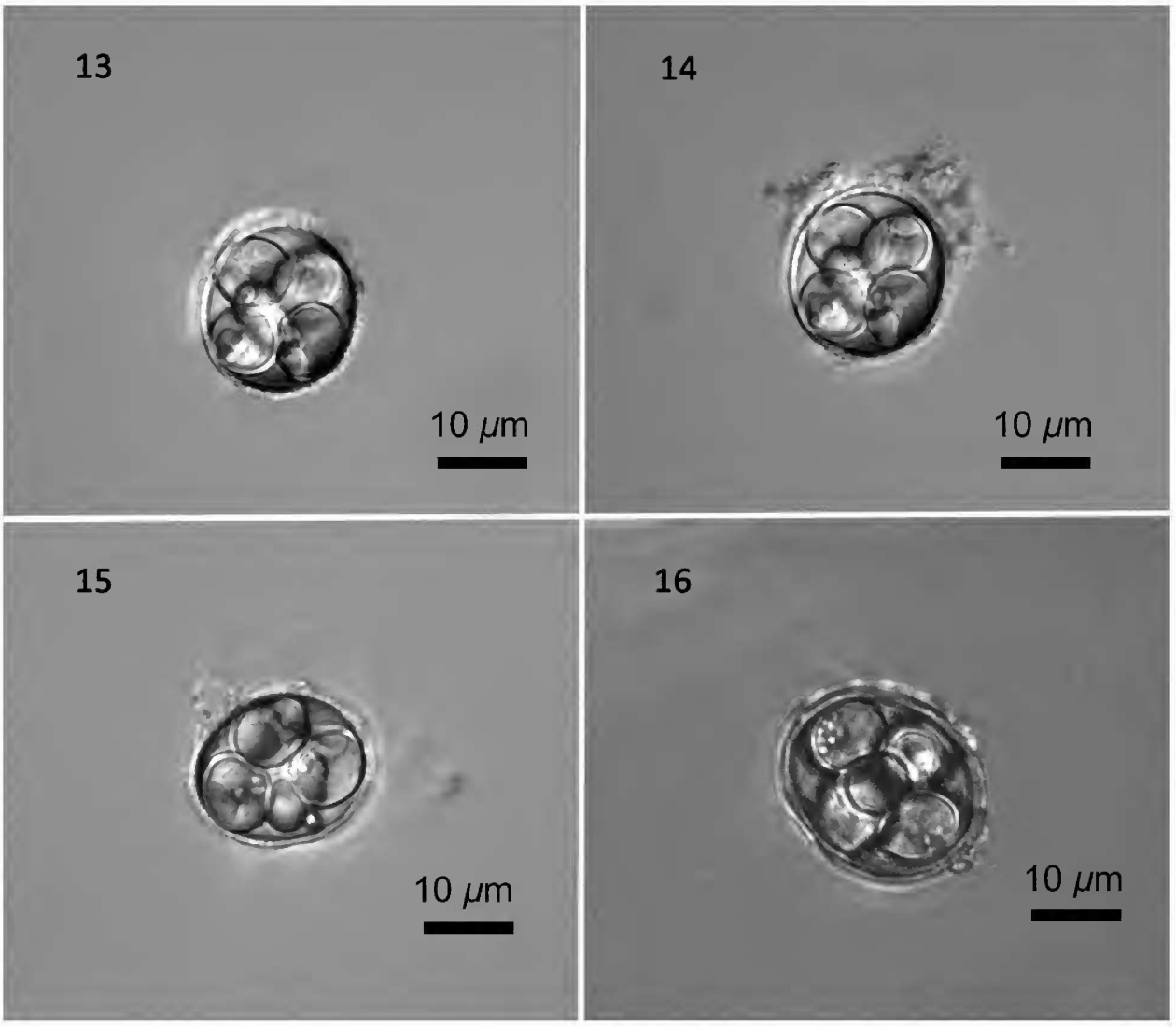
Specimens deposited.—NP1322 and NP1466.

Location.—Intestine. Oocysts obtained from fecal sample.

Locality.—Infected Plains Pocket Mice were trapped on two occasions: 31 July 2012 [Arapaho Prairie] (41°29′19″N; 101°51′30″W); 1 August 2013 [Ackley Valley, Haythorn Ranch, Arthur County, NE] (41°12′00″, 101°26′06″W).

Canonical Discriminant Analysis

Results of the canonical discriminant analysis of mensural characters of the coccidians studied are shown in Figure 17. For this analysis, centroids of all species were found to be significantly different, with 88% of the variation in the data being accounted for in the first two canonical variates. Figure 17 shows a plot of discriminant scores showing the minimum polygons enclosing the spread of individuals for each species of *Eimeria* identified in this study.



Figures 13–16. Photomicrographs of poorly sporulated oocysts of an unidentified species of *Eimeria* recovered from the feces of *Perognathus flavescens*.

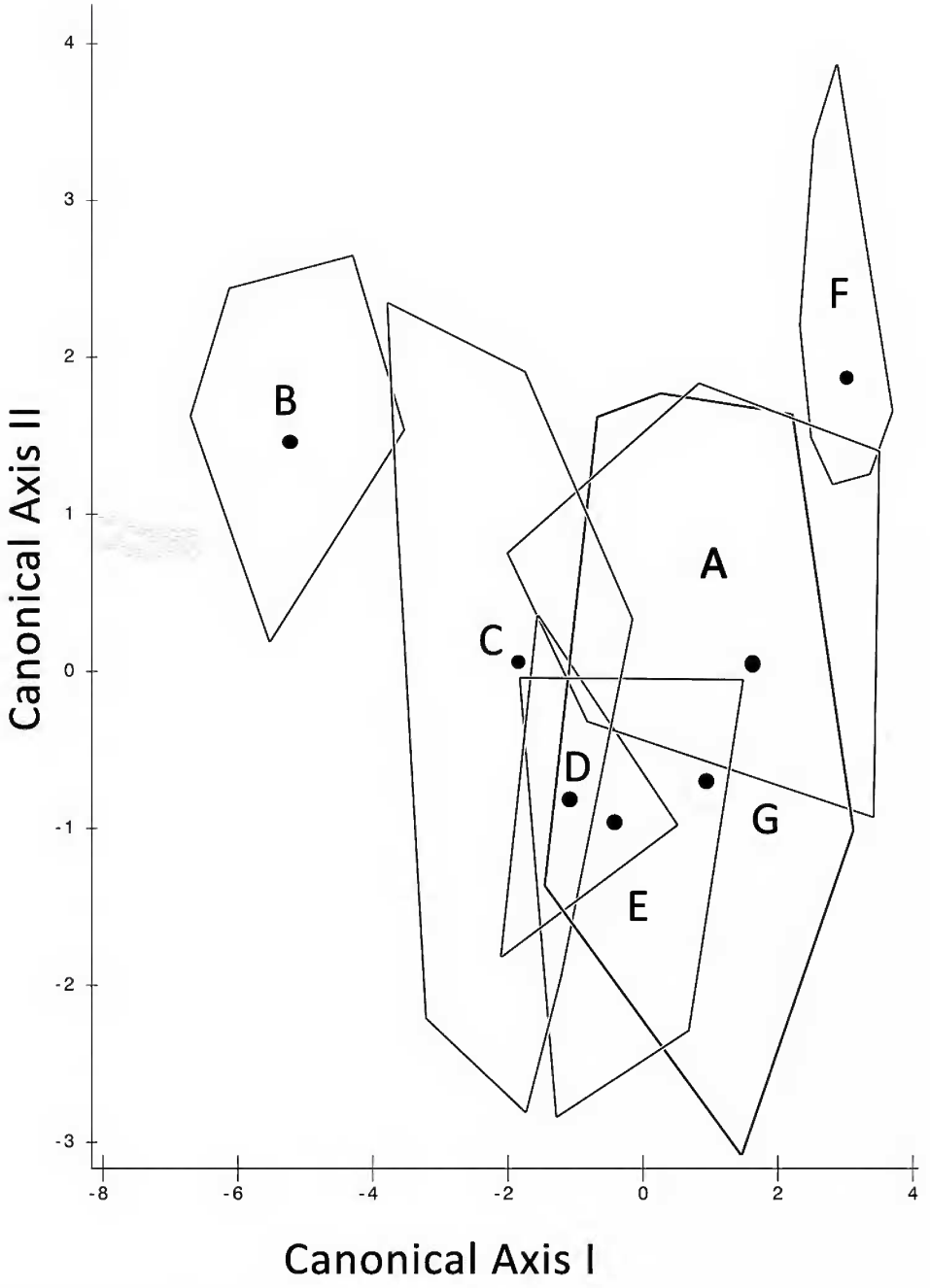


Figure 17. Ordination showing minimum polygons for each of the host species from the first two canonical axes derived from the morphometric analysis of *Eimeria* from rodents collected from western Nebraska. Polygon labels are as follows: A = *Eimeria arizonensis* in *Peromyscus maniculatus*; B = *Eimeria balphae* in *Dipodomys ordii*; C = *Eimeria onychomysis* in *Onychomys leucogaster*; D = *Eimeria hispidensis* in *Chaetodipus hispidus*; E = *Eimeria* sp. in *Perognathus flavescens*; F = *Eimeria arizonensis* in *Peromyscus leucopus*; and G = *Eimeria arizonensis* in *Reithrodontomys megalotis*. The multivariate centroid of each group is represented by a black dot.

DISCUSSION

Some species of *Eimeria* have been shown to exhibit varying levels of both phenotypic plasticity and assumed host specificity (Wash et al. 1990). In our study, *E. arizonensis* was found in individuals of *Peromyscus leucopus*, *P. maniculatus*, and *Reithrodontomys megalotis*. It has been observed previously that *E. arizonensis*-like oocysts can infect these three species, among others (Wash et al. 1990; Upton et al. 1992). Additionally, it has been observed that these *E. arizonensis*-like oocysts have shown phenotypic plasticity that now is accepted as a well-documented case of host-induced variation that is manifested both morphologically and biochemically [see review by Joyner 1982 and more specific information on this topic in Duszynski (1971) and Wash et al. (1990)]. With the exception of *E. arizonensis*, all coccidians studied here showed very similar mensural data compared to their descriptions. One host of *E. arizonensis* harbored oocysts that were on average 7 x 3 micrometers (length and width, respectively) larger than the means given in the published descriptions, but all other characters agree with these descriptions.

The wide range of phenotypic plasticity demonstrated by *E. arizonensis* (Upton et al. 1992) appears to indicate that this may be a cryptic species of *Eimeria* that may be morphologically convergent in different hosts with overlapping habitats. To understand this system, we suggest that molecular genetic studies be conducted and, in fact, a new method of generating genetic data from fewer than five oocysts has just been published (Gerhold et al. 2015). Coinfections are also difficult to detect when dealing with field-captured wildlife. Gardner and Duszynski (1990) list some of the difficulties associated with the phenotypic plasticity of eimerian oocysts in subterranean rodents (Ctenomyidae in Bolivia) and these authors show that care must be taken in determination of species in these cases.

The multivariate statistical analysis (plot shown in Fig. 17) shows moderate separation of the species considered herein. *Eimeria balphae* from *Dipodomys ordii* and *E. arizonensis* from *Peromyscus leucopus* show strong separation, mostly due to overall size of the oocyst and thickness of oocyst wall. The other eimerians appear to have a significant amount of overlap in multivariate space, indicating that these oocysts have very similar morphological characteristics. This further illustrates the difficulty of identifying species of *Eimeria* using only morphology of oocysts. Again, genetic work should be done to enhance this study (see Zhao and Duszynski 2001; Gerhold et al. 2015).

A common problematic host/parasite association has appeared in the case of the eimerian parasites from *Perognathus flavescens*. Individual oocysts from this host were not well-sporulated and therefore we were unable to obtain much useful information from study of these specimens. Further investigation of these specimens could lead to a description of a new species of *Eimeria* because, to our knowledge, no previous papers relative to species of *Eimeria* from *P. flavescens* have been published. Presently, we do not have sufficient data to properly identify and describe these oocysts.

One potential explanation for the annual difference in prevalence observed in this study is the amount of rainfall that occurred in the area. Rainfall data were obtained from a meteorological station at CPBS run by the High Plains Regional Climate Center (Anonymous 2015). June and July 2012 was markedly dry, with rainfall amounts only 19% of average for that period. The summer of 2013, though still abnormally dry at 49% of the mean precipitation, received more than twice as much rainfall. During this same time period, prevalence of *Eimeria* tripled. Data from the Sevilleta LTER site in New Mexico (Gardner, unpublished) suggests that precipitation and prevalences of parasites are directly linked. These data show that parasites that are transmitted directly from host to host (without an intermediate host), such as *Eimeria*, have prevalences that wax and wane in correlation with fluctuating rainfall measures with little to no notable lag, whereas parasites that utilize intermediate host(s) appear to follow the same pattern set by precipitation, but with a delay of about one year. These findings support the prevalence differences observed in this study. In addition, high precipitation levels in 2015 would indicate that an increase in coccidian prevalences can be expected in 2015 with a concomitant increase in helminth prevalence in 2016. Further study over many years would be beneficial to the understanding of prevalence shifts. The summer of 2014 is of particular interest, as there was a recorded rainfall amount (at the CPBS station) of 217% of the average in the area (Anonymous 2015).

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